

Research Article

A 12S rDNA barcode reference library for Neotropical electric fishes (Teleostei, Gymnotiformes)

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Abstract

DNA barcodes are instrumental for identifying species from tissues and environmental samples. Metabarcoding-based assessments of biodiversity depend on taxonomically accurate barcode sequence libraries that can be queried to determine which species are present in a sample. The mitochondrial 12S ribosomal RNA gene has been shown to function as a robust barcode for differentiating taxa and identifying species using environmental DNA (eDNA), but complete 12S reference barcodes remain scarce in public databases. We assembled a library of 12S rDNA reference barcodes for 152 species of Neotropical electric fishes from the order Gymnotiformes (Teleostei, Ostariophysi). These fishes are an abundant and diverse component of Neotropical freshwater ecosystems, but are often difficult to collect through conventional sampling approaches because of their nocturnal activity and association with dense substrates or deep-water habitats. This makes eDNA metabarcoding an especially suitable approach for their detection. We compiled a dataset comprising all gymnotiform families, with most sequences covering almost the entire 12S gene. Across the gene and within two widely used 12S eDNA metabarcoding loci (MiFish and teleo), our sequences show sufficient interspecific divergence to discriminate the majority of knifefish species included in our analyses. This dataset, therefore, provides a valuable new set of resources for differentiating amongst gymnotiform species and identifying them in environmental DNA samples.

Key words: 12S, barcoding, eDNA, Gymnotiformes, knifefish, metabarcoding, Neotropics

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Introduction

Molecular methods of species identification are increasingly important tools for ecosystem conservation and management, particularly for threatened and endangered species. Environmental DNA (eDNA) collected from water, sediment or soil samples provides a non-invasive means of characterising the biological composition of habitats and monitoring biodiversity (Thomsen and Willerslev 2015; Miya 2022). In aquatic systems, eDNA-based monitoring typically involves collecting water samples from the habitat of interest and using metabarcoding to infer which species have been present and shed DNA into the



surrounding water (Deiner et al. 2017). The reliability of such metabarcoding surveys depends critically on the availability of robust, taxonomically-verified genetic barcodes that can be used to identify sequences from samples of unknown composition (Hubert and Hanner 2015; Thomsen and Willerslev 2015). Reference barcode libraries with wide taxonomic and geographic breadth are, therefore, increasingly important for conservation biologists and taxonomists, facilitating the discovery of new species in addition to the interpretation of environmental genetic data (Ahmed et al. 2025; Jiménez-Segura et al. 2025). Unfortunately, barcode coverage still remains limited for many taxa in megadiverse tropical regions (Marques et al. 2021; Altermatt et al. 2025).

The Neotropical electric knifefishes (order: Gymnotiformes) comprise more than 250 species distributed across Central and South America, with peak diversity in the Amazon and Orinoco River Basins (Crampton 2011; Crampton and Poon 2024). Gymnotiformes, also referred to as Neotropical knifefishes, employ electrosensory systems to sense their environment and communicate (Crampton 2019). Their ecological breadth, spanning a wide range of habitat types, makes Gymnotiformes an informative group for assessing biodiversity and ecosystem integrity in South American river systems (Albert and Crampton 2005; Crampton 2011). At the same time, many species inhabit deep channels or hard-to-sample substrates and are nocturnally active, complicating conventional survey methods (Mago-Leccia 1994; Ford and Albert 2022). These characteristics make eDNA analysis an especially suitable approach for detecting gymnotiform fishes; in turn, eDNA offers the potential to standardise biodiversity assessments and improve delineation of species' geographic ranges (Rees et al. 2014).

The 12S ribosomal RNA gene (12S hereafter) is a widely used mitochondrial DNA barcode with several properties that make it particularly effective for species identification. As with other mitochondrial genes, it has a higher cellular copy number and is, therefore, more abundant in environmental samples than most nuclear genes (Dziedzic et al. 2023). The relatively rapid evolutionary rate of mitochondrial genes also provides finer taxonomic resolution than many nuclear genes (Allio et al. 2017). Previous barcoding efforts for Gymnotiformes have focused primarily on the cytochrome c oxidase I (COI) gene (Janzen et al. 2022). 12S has similarly been validated as an effective barcode gene in a range of taxa (Chan et al. 2022) and is now widely used in aquatic eDNA studies to identify fish species and corroborate results obtained from other barcode markers (Van Nynatten et al. 2023; Stoeckle et al. 2024). Comparative assessments further indicate that 12S often outperforms other mitochondrial genes in metabarcoding approaches for fishes (Zhang et al. 2020). Despite these advantages, 12S remains under-used in freshwater fishes, particularly within the Gymnotiformes, where only 38 species have 12S sequences available on GenBank. Many of these existing records also precede major taxonomic revisions of the gymnotiform order. The diversity of this order has expanded substantially in recent history, with nearly 100 species described in the past 15 years (Fricke et al. 2025), raising concerns that older barcodes may correspond to outdated or incorrect species identifications. A comprehensive and up-to-date set of complete 12S sequences is, therefore, needed to support reliable identification of Neotropical electric fishes.

Here, we present a reference library of nearly full-length 12S rDNA sequences for 152 gymnotiform species. All specimens had up-to-date species identifications and were selected, where possible, based on their proximity to their

respective type localities. We designed multiple primer sets to amplify a locus spanning most of the 12S gene across the major lineages of Neotropical electric fishes, yielding sequences that encompass all gymnotiform families and 30 of the 35 genera in the order. Our library includes four times as many taxa as currently represented by 12S sequence data on GenBank. To assess the resolving power of this marker, we quantified sequence divergence amongst closely-related species and found that this 12S fragment is sufficient to distinguish the great majority of gymnotiform taxa. Finally, we examined sequence similarity in two common metabarcoding regions of the 12S gene and found that almost all species included in our library can be uniquely identified using these gene fragments. These results ultimately support the use of 12S as a metabarcoding gene for eDNA-based identification of Gymnotiformes.

Materials and methods

Specimen selection

We obtained 13 specimens from their species' type locality, 18 paratypes and one holotype. When selecting the remaining 120 specimens to sequence for reference barcodes, we prioritised using samples from specimens in close proximity to each species' type locality. In almost all cases, tissues were obtained from museum voucher specimens (see Suppl. material 1). All specimens were examined and identified morphologically using the original species descriptions as diagnostic references. Tissue samples were preserved in 95% ethanol and stored at -20 °C.

DNA extraction and PCR

DNA was extracted using QIAGEN DNeasy Blood and Tissue kits following the manufacturer's protocol and stored at -20 °C. To PCR-amplify a fragment containing nearly the full length of the 12S gene, we developed several primer sets tailored to different gymnotiform genera and families (Table 1). Most species were successfully amplified using the primer pair of Van Nynatten et al. (2023): 12SteleoFull F1 (5' – CAAAGGCTTGGTCCTGAC – 3') and 12SteleoFull R (5' – AGCATTCCCTTGCGGTAC – 3'). In Gymnotiformes, this primer pair targets an approximately 1130 bp amplicon that spans almost the entire 12S gene and extends into the adjacent tRNA-Val and 16S rRNA genes. For taxa that did not amplify with this pair, we used more taxon-specific primers designed to target amplicons that likewise encompass the majority of the 12S gene (Table 1). PCR reactions consisted of 2 µl of 10X PCR Buffer (BIO-HELIX Co., Ltd), 0.5 µl of 2.5 mmol/l dNTP mix (FroggaBio Inc.), 0.4 µl each of 10 µmol/l forward and reverse primer, 0.2 µl of 5U/µl Taq DNA polymerase (FroggaBio Inc.), 2 µl of extracted DNA and 14.5 µl of nuclease-free water (Sigma-Aldrich Co.) for a final reaction volume of 20 µl. Thermocycling began with a 4 min heating phase at 95 °C, followed by 35 cycles of 95 °C denaturing for 30 s, 52 °C annealing for 30 s and 72 °C extension for 45 s. These cycles were followed by a final 5 min extension phase at 72 °C. Amplification success was verified by electrophoresing 5 µl of DNA product mixed with 1 µl of 6x loading buffer (FroggaBio Inc.), run on a 1% agarose gel stained with 4 µl of RedSafe nucleic acid staining solution (FroggaBio Inc.).

Table 1. Primer sequences used to amplify fragments containing nearly the full length of the 12S gene, along with the genera or families they were designed to target. Sequences for the teleo R A and teleo R B reverse primers were adapted from Thomsen et al. (2016).

Forward Primers		
Primer	Sequence (5'-3')	Target Group
12SteleoFullL F1	CAAAGGCTTGGTCCTGAC	Gymnotiformes
G12SRha F	CAAAGGTCTGGTCCTGGC	Rhamphichthyidae
Eel F	CAAAGGTTTGGTCCTAGCC	<i>Electrophorus</i>
Hyp/Apt F	CGTGAGAATGCCCTCAA	Hypopomidae and Apterodontidae
Gym F	GTGAGAATGCCCCAAAACCTT	<i>Gymnotus</i>
Ste F	CCCAAGACGCCTTGCTA	Sternopygidae
Reverse Primers		
Primer	Sequence (5'-3')	Target Group
12SteleoFullL R	AGCATTCCTTGCGGTAC	Gymnotiformes
teleo R A	CTTCCGGTACACTTACCATG	Gymnotiformes
teleo R B	CTTCCGGTGCGCTTACCATG	<i>Gymnorhamphichthys</i>
Hyp R	TTCTCGGTGTAAGGGAGAT	<i>Hypopomus</i>
Gym/Hyp R	AGTGCACCTTCCGGTAC	<i>Gymnotus</i>
Apt R	CTGGTTGTTCCAAGTACAC	Apterodontidae
Brachy R	GCATGAATGTCTTCTCGGT	<i>Brachyhypopomus</i>
Ste R	TCTCGGTGTAAGGGAGAT	Sternopygidae

Sequencing

PCR products were purified using ExoSAP-IT PCR product clean-up reagent (ThermoFisher Scientific Inc.). Forward and reverse primers were then added to purified PCR products and sent to The Centre for Applied Genomics (Toronto) for Sanger sequencing. Forward and reverse reads were assembled and trimmed in Geneious Prime (Geneious 2024.0.5) to generate a consensus sequence for each species. The average sequence length after trimming was 985 bp.

Collection of existing barcode data

To compare the taxonomic coverage of our reference barcode library with publicly available gymnotiform 12S sequences, we retrieved 12S fragments from GenBank using the PhylotaR package in R (Sanderson et al. 2008; Bennett et al. 2018; R Core Team 2025). We also downloaded complete mitochondrial genomes containing 12S, as well as any additional 12S fragments for gymnotiform species not captured by PhylotaR. Sequences with unverified species identifications were excluded.

Analysis of new 12S sequences

Alignments of the newly-generated, nearly full-length 12S sequences were generated using the MUSCLE algorithm in AliView (Edgar 2004; Larsson 2014). To better visualise relationships amongst taxa and confirm the taxonomic placements of our specimens, we reconstructed a Maximum-Likelihood tree using PhyML3.0 (Guindon et al. 2010). Outgroup sequences belonging to *Gonorynchus abbreviatus*, *Ictalurus punctatus* and *Astyanax mexicanus* were obtained from mitochondrial genomes in GenBank (Suppl. material 2). Pairwise sequence divergence amongst

gymnotiform species was quantified with p-distances calculated from the full alignment and from the smaller barcoding loci within it using MEGA12 (Tamura et al. 2004; Kumar et al. 2024). For each species, we determined the smallest distance to another species, referred to below as minimum sequence divergence.

Results

Taxonomic coverage of barcode sequences

Our new barcode library spans 30 genera and 152 species of Gymnotiformes. By comparison, only 38 gymnotiform species currently have verified 12S sequence data on GenBank, with 25 of these species being represented only by smaller fragments of the gene (see Suppl. material 2). Additionally, more than half of the GenBank records correspond to just one genus: *Electrophorus*. The reference library presented here adds 122 additional gymnotiform species: 23 Gymnotidae, 25 Hypopomidae, 17 Rhamphichthyidae, 36 Aptereronotidae and 21 Sternopygidae – thereby increasing by fourfold the number of gymnotiform species with 12S sequence data. All new sequences generated for this study have been deposited in GenBank (see Suppl. material 1).

Phylogenetic tree

The phylogenetic relationships inferred from our 12S barcode sequences broadly match established hypotheses of gymnotiform systematics (Fig. 1). Most genera form monophyletic groups and the few species that fall outside of their expected generic clades remain in close proximity to them. At the family level, four of the five families are recovered as monophyletic. The exception is Sternopygidae, which appears polyphyletic: *Sternopygus* clusters nearer to Gymnotidae, Rhamphichthyidae and Hypopomidae, whereas the remaining five sternopygid genera group more closely with Aptereronotidae. This pattern reflects the limited power of a single mitochondrial marker for resolving deeper phylogenetic relationships (Cao et al. 1994; Tagliacollo et al. 2016).

Genetic distances between barcode sequences

The ability of the 12S barcodes to distinguish closely-related gymnotiform species depends on the amount of pairwise sequence divergence between taxa. Across the library, the mean minimum pairwise divergence between species was 2.91% (Table 2). The smallest observed divergence between species was 0%, observed for *Sternarchella duccis* and *Sternarchella orthos*, and for *Gymnotus varzea* and *Gymnotus chaviro* (Table 2). Fourteen additional species pairs, as well as a cluster of five *Sternarchella* species, showed less than 1.0% divergence (see Suppl. material 3).

Sequence diversity in common 12S eDNA barcoding regions

To evaluate the suitability of 12S for metabarcoding Gymnotiformes, we examined sequence variation within two loci commonly targeted in eDNA studies (Fig. 2). The MiFish-U primer set, originally developed to amplify a ~ 170 bp amplicon

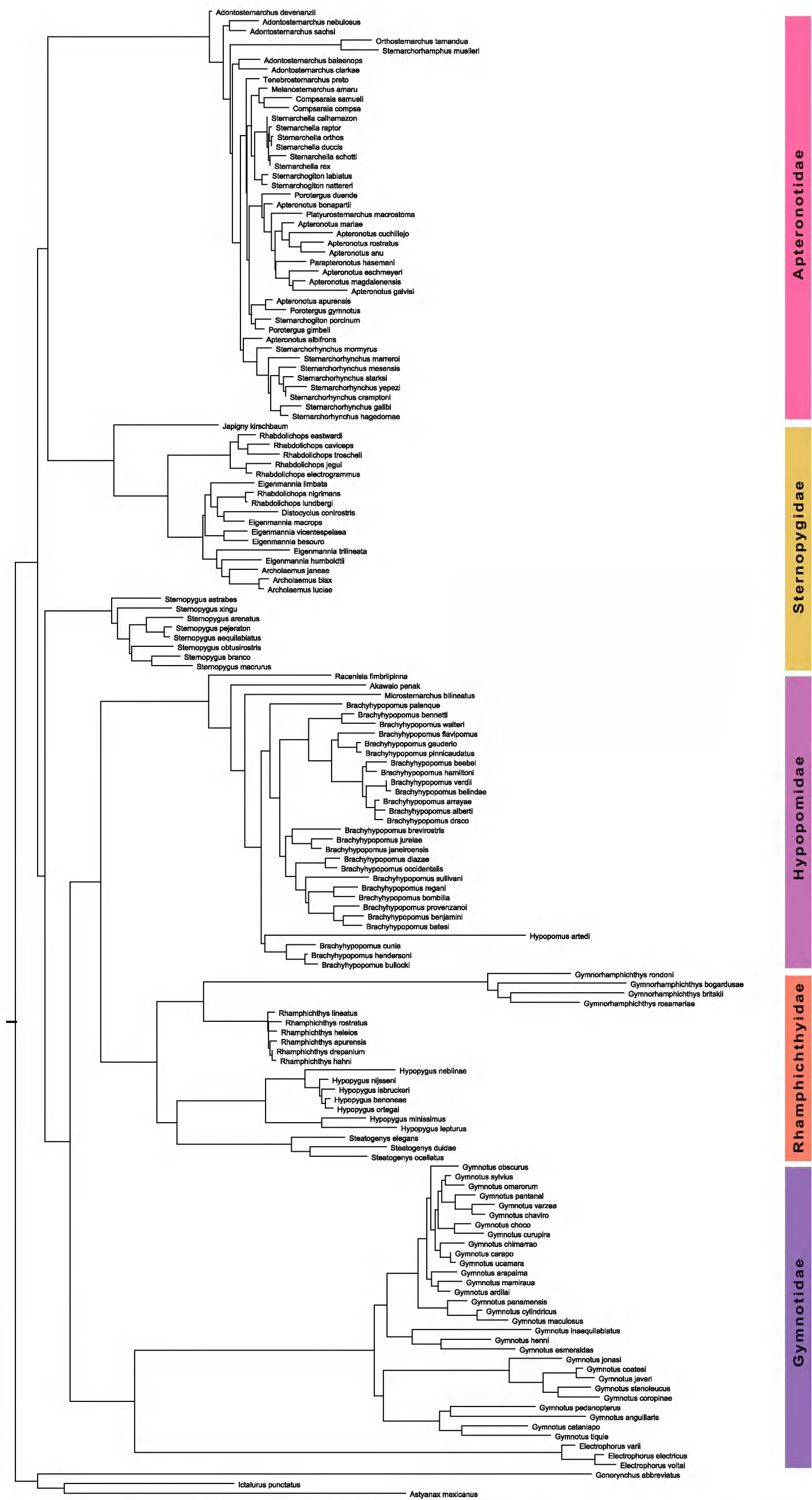


Figure 1. Maximum-Likelihood 12S gene tree generated from sequences in a new barcode reference library. Coloured bars indicate the positions of gymnotiform families along the tree.

Table 2. Summary of sequence length, divergence and identity for the 12S reference barcodes and for three commonly-used barcoding regions. Average minimum pairwise sequence divergence for each locus was calculated using p-distances. Metrics for the MiFish barcode and our reference barcodes were calculated using the complete library of 152 species. Metrics for the teleo-barcode were based on 132 species in the library with complete sequences in the region. For comparison, COI data were taken from Janzen et al. (2022) and encompass 167 species/sequences.

Locus	Average Length	Average Minimum Sequence Divergence	Number of Pairs with 0% Divergence
12S Reference Barcodes	985	2.91%	2
12S MiFish Barcode	171	3.17%	15
12S teleo Barcode	62	6.62%	26
COI Barcode	640	6.36%	2

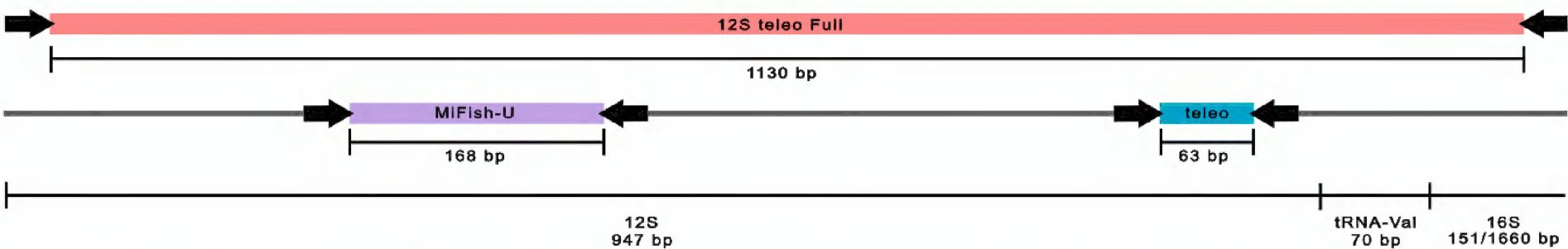


Figure 2. Primer and amplicon locations for reference barcodes containing the majority of the 12S gene and smaller eDNA barcodes, shown relative to the *Brachyhypopomus occidentalis* mitochondrial genome. Adjacent tRNA-Val and 16S rRNA genes are indicated. Approximate primer binding sites are marked with black arrows. The ~ 1130 bp region targeted by the 12S teleo Full primers (used for the majority of sequences in the barcode reference library) spans nearly the entire 12S gene and extends into the neighbouring tRNA-Val and partial 16S rRNA genes. The MiFish-U and teleo-targeted metabarcoding regions, along with their primer binding sites, are nested within the 12S gene.

in marine fish species, is widely recognised as one of the most versatile primer pairs for fish metabarcoding due to the highly variable region it targets (Miya et al. 2015; Miya et al. 2020). In our library, interspecific divergence within this region was sufficient to distinguish most gymnotiform species. Nevertheless, 15 species pairs could not be distinguished from one another using this barcode alone due to complete sequence identity at the locus (Tables 2, 3). These cases indicate that, for some taxa, MiFish-U may need to be complemented with additional loci to achieve unambiguous species identifications.

Another widely-used primer pair designed to target 12S is teleo_F and teleo_R (Valentini et al. 2016). Previous work comparing the teleo-amplified region to the MiFish barcoding region has suggested that, in cases where the MiFish amplicon has insufficient taxonomic resolution, the approximately 65 bp fragment amplified by teleo_F and teleo_R can sometimes resolve species that are otherwise indistinguishable (Fig. 2) (Polanco et al. 2021). To determine if this was also the case for Gymnotiformes, we examined sequence diversity in the teleo-region for the 15 species pairs that were identical at the MiFish locus. Incorporating the teleo-amplicon resolved 10 of these 15 species pairings (Table 3), each of which showed detectable sequence divergence at this locus. Thus, for most taxa in our dataset, the teleo-targeted region of the 12S gene restores species-level resolution when MiFish alone is insufficient. The teleo-locus by itself, however, contains 12 species pairs or groups with complete sequence identity. Used in combination, these two common barcoding regions can, therefore, yield correct species identifications for the majority of Gymnotiformes. The remaining indistinguishable species pairs warrant further investigation using alternative genetic markers.

Table 3. Species pairs with identical MiFish barcode sequences and the loci capable of distinguishing them. Check marks indicate loci showing sequence differences between species. COI barcode data are from Janzen et al. (2022).

Species Pairs with Identical MiFish Barcodes	12S teleo Barcode	12S Reference Barcode	COI Barcode
<i>G. arapaima</i> <i>G. ucamara</i>		✓	✓
<i>G. varzea</i> <i>G. chaviro</i>			
<i>H. nijsseni</i> <i>H. isbruckeri</i>		✓	✓
<i>R. nigrimans</i> <i>R. lundbergi</i>	✓	✓	✓
<i>S. orthos</i> <i>S. duccis</i>			
<i>S. duccis</i> <i>S. labiatus</i>	✓	✓	✓
<i>S. orthos</i> <i>S. labiatus</i>	✓	✓	✓
<i>P. duende</i> <i>S. nattereri</i>	✓	✓	✓
<i>P. duende</i> <i>S. rex</i>	✓	✓	✓
<i>P. duende</i> <i>S. calhamazon</i>	✓	✓	✓
<i>S. nattereri</i> <i>S. rex</i>	✓	✓	✓
<i>S. nattereri</i> <i>S. calhamazon</i>	✓	✓	✓
<i>S. rex</i> <i>S. calhamazon</i>		✓	✓
<i>B. verdii</i> <i>B. belindae</i>	✓	✓	✓
<i>B. bullocki</i> <i>B. hendersoni</i>	✓	✓	✓

Conclusions and discussion

A comprehensive reference library of 12S rDNA sequences for Gymnotiformes

Our newly-assembled gymnotiform 12S reference library provides a comprehensive and taxonomically broad resource for the molecular identification of Neotropical electric fishes. This library comprises 152 species of Gymnotiformes, spanning all families and almost all genera. The six genera not represented here each contain fewer than three species, many with restricted geographical ranges and, together, account for only a small fraction of overall gymnotiform diversity (Fricke et al. 2025). The breadth of taxonomic coverage, together with the near-full-length 12S sequences obtained for each specimen, make this the most complete compilation of 12S data for Neotropical knife-fishes to date. Although additional species remain to be sampled, the diversity represented in this library should allow the identification of unknown taxa to at

least the genus level. As such, this resource will be valuable for the taxonomic classification of unknown gymnotiform tissues and eDNA-derived sequences.

The average minimum pairwise divergence between species in our 12S reference barcode library was 2.91% (Table 2). Most species pairs in our reference library have > 1.0% divergence. There were several species pairs with < 1.0% divergence in their 12S barcode sequences, including two with complete sequence identity. In comparison, COI barcodes for Gymnotiformes have an average minimum sequence divergence of 6.36% (Table 2; Janzen et al. (2022)). This is consistent with previous comparisons, which have shown that 12S sequences tend to have smaller interspecific genetic divergences on average than COI (Milan et al. 2020; Wang et al. 2023). These patterns indicate that species-level thresholds for 12S barcodes in Gymnotiformes should be lower than those typically applied to COI, where a 2.0% threshold for interspecific divergence is often used for fishes (Ward 2009).

Our focus on interspecific divergence, rather than intraspecific diversity, prevents us from quantifying a 12S “barcoding gap” for Gymnotiformes in this study. Future sequencing of additional specimens should allow this gap to be determined. Future barcoding efforts should additionally focus on sampling individuals from across species’ geographic distributions to ensure availability of molecular reference data for distinct lineages of the same species (Gaytán et al. 2020). Assessment of intraspecific variation is important for Gymnotiformes, as some species in this order include complexes of genetically divergent lineages that require more extensive sampling across localities for comprehensive representation (Milhomem et al. 2008; de Santana and Vari 2013). Nevertheless, the 12S sequences generated here contain sufficient variation to enable identification of the majority of gymnotiform species from unknown samples and serve as a reliable foundation for future barcoding studies of these fishes.

eDNA and metabarcoding studies using the 12S gene

Environmental DNA recovered in metabarcoding studies is often highly fragmented (Thomsen and Willerslev 2015). Metabarcoding primer sets, therefore, target short, highly variable regions of barcoding genes (Milan et al. 2020; Miya et al. 2020). In this study, we examined sequence diversity within the two most widely-used 12S metabarcoding loci: MiFish-U and teleo. Across the full dataset, only five gymnotiform species pairs could not be differentiated using either of these barcodes: *Gymnotus arapaima* – *Gymnotus ucamara*, *Gymnotus varzea* – *Gymnotus chaviro*, *Hypopygus nijsseni* – *Hypopygus isbruckeri*, *Sternarchella orthos* – *Sternarchella duccis* and *Sternarchella rex* – *Sternarchella calhamazon*. Four of these species pairs have either partial or no overlap in their known distributions, meaning their identities could potentially be resolved using the localities of samples (Albert et al. 2005; Crampton et al. 2005; Maxime and Albert 2009; Crampton 2011; Peixoto et al. 2013; Evans et al. 2017). The remaining species pair, *Sternarchella rex* and *Sternarchella calhamazon*, unfortunately has overlapping distributions, meaning locality information would not aid in species discrimination (Evans et al. 2017). Ultimately, the inability of the MiFish and teleo-loci to discriminate between some gymnotiform species could reflect the intrinsic taxonomic resolution of this group. Most of these indistinguishable pairs also show minimal

divergence in COI, a more commonly used mitochondrial barcode (Janzen et al. 2022), suggesting that their mitochondrial genomes may lack sufficient variation for reliable species-level assignment using barcode markers alone. These cases, therefore, likely represent the practical boundary of what can be resolved in gymnotiform eDNA studies using current metabarcoding loci.

For the remaining Gymnotiformes in our library, the two commonly used 12S metabarcoding loci provide reliable species-level discrimination, particularly when used together. In most cases, both the MiFish and teleo-loci are unique to individual species, and species that share complete sequence identity at one locus almost always differ at the other. Accordingly, we recommend employing both barcodes when possible to minimise ambiguity in species assignments. Multiplex metabarcoding approaches, in which multiple markers are used simultaneously to improve taxonomic resolution, have already been shown to increase the proportion of correctly identified species in eDNA studies (Zhang et al. 2018; Eastwood et al. 2024). Using both 12S loci together, therefore, represents a promising strategy for comprehensive detection of gymnotiform biodiversity and can enhance efforts to document species distributions and community composition in Neotropical freshwater ecosystems.

Our library of nearly full-length 12S sequences also provides a valuable resource for developing new metabarcoding primers. The 12S rRNA gene contains substantial variation, especially in the loop regions of the rRNA, where bases are less constrained by base pairing in the molecule's secondary structure (Wang and Lee 2002). The two commonly targeted metabarcoding regions, therefore, represent only a subset of the potentially informative loci within this gene. The near-complete 12S sequences generated here can be used to identify additional highly variable regions suitable for future primer design in metabarcoding studies.

Importance of molecular conservation approaches for neotropical electric fishes

The 12S reference library presented here offers a molecular foundation for assessing gymnotiform diversity across the Neotropics. Thorough sampling of Gymnotiformes often requires dedicated nocturnal effort and, in some habitats, logistically demanding methods such as deep-water trawling (Mago-Leccia 1994; Crampton 2011; Ford and Albert 2022). Metabarcoding reference data, therefore, has the potential to greatly enhance our knowledge of gymnotiform occurrences by providing an alternative method for detecting these fishes (Fraija-Fernández et al. 2020). These approaches additionally have the potential to reveal habitat shifts and dispersal dynamics associated with seasonal flood regimes and other environmental disturbances (Carvalho et al. 2024; Fan et al. 2025). Ultimately, eDNA-based metabarcoding of Neotropical electric fish populations using this 12S reference library can greatly improve our understanding of how these species are distributed throughout their ecosystems.

Beyond basic presence-absence information, eDNA-based mapping of gymnotiform distributions can reveal patterns of species co-existence, interaction and electric signal diversity (Crampton 2011; Gu et al. 2023; Hauser et al. 2024; Boyse et al. 2025). eDNA has previously been used, for example, to measure site occupancy over time for estuarine fish metapopulations (Martel et al. 2021), an

approach that is increasingly relevant in the Amazon as habitat degradation accelerates (Herrera-R et al. 2020; Lapola et al. 2023). Metabarcoding analyses of species co-occurrence can likewise reveal predator-prey interactions that may have gone undiscovered without environmental genetic data (Riaz et al. 2023). For Gymnotiformes, reference barcodes for eDNA studies may, therefore, enable us to identify or confirm predatory interactions amongst species. Taken together, genetic approaches can provide us with a better understanding of gymnotiform distributions and dynamics and are of growing importance given the ecological and environmental challenges faced by Neotropical ecosystems. As Neotropical knifefishes are both diverse and abundant across a wide range of habitats (van der Sleen and Albert 2018), metabarcoding studies of gymnotiform assemblages could serve as sensitive indicators of broader ecological responses to climate change and human disturbance. The 12S reference library presented here, therefore, provides a molecular framework that can support robust monitoring and conservation assessments for Gymnotiformes and the threatened Neotropical ecosystems they inhabit.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

No use of AI was reported.

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Author contributions

Conceptualisation: WGRC, NRL. Data curation: NDJ, WGRC. Formal analysis: NDJ. Funding acquisition: NRL, WGRC. Investigation: NDJ. Methodology: NDJ. Project administration: NRL. Resources: WGRC, NRL. Supervision: NRL. Visualisation: NDJ. Writing – original draft: NDJ. Writing – review and editing: NDJ, WGRC, NRL.

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Data availability

All sequences have been deposited to GenBank under accession numbers [PZ025974–PZ026125](#).

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Supplementary material 1

Supplementary information 1

Authors: Nicole D. Juby, William G. R. Crampton, Nathan R. Lovejoy

Data type: xlsx

Explanation note: Species, localities and type statuses of specimens used for the 12S reference barcode library.

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Link: <https://doi.org/10.3897/mbmg.10.181378.suppl1>

Supplementary material 2

Supplementary information 2

Authors: Nicole D. Juby, William G. R. Crampton, Nathan R. Lovejoy

Data type: xlsx

Explanation note: Species and accession numbers of full-length and partial 12S sequences currently available for Gymnotiformes on GenBank and species and accession numbers of outgroup mitochondrial genomes used in phylogenetic analysis.

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Supplementary material 3

Supplementary information 3

Authors: Nicole D. Juby, William G. R. Crampton, Nathan R. Lovejoy

Data type: xlsx

Explanation note: Distance matrix (p-distances) of 152 gymnotiform 12S sequences.

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Link: <https://doi.org/10.3897/mbmg.10.181378.suppl3>